STABILIZED THYROXINE COMPOUNDS

CROSS RELATED APPLICATION

[001] This application claims priority to and is a continuation-in-part of U.S. Application 09/440,635 filed on November 16, 1999 which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[002] This invention relates to the use of a protecting group to stabilize thyroxine and related compounds, thus extending the shelf life of the drugs.

BACKGROUND OF THE INVENTION

[003] Thyroid hormones T4 and T3 play a crucial role in metabolic homeostasis and affect the function of virtually every organ system. In healthy individuals, serum concentrations of the thyroid hormones are controlled by a classic negative-feedback system involving the thyroid gland, the pituitary gland, the hypothalamus and peripheral tissues, such as the liver. In response to the thyroid-stimulating hormone (TSH; also known as thyrotropin) produced by the pituitary, the thyroid gland normally releases an estimated 70 to 90 mcg of T4 and 15 to 30 mcg of T3 into the blood stream per day. Although T3 is secreted by the healthy thyroid, the major portion of T3 in circulation is thought to result from deiodination of T4 by peripheral tissues, particularly the liver. Synthesis and release of TSH by the pituitary is stimulated by thyroid-releasing hormone (TRH) a tripeptide produced by the hypothalamus in response to changes in metabolism caused by low levels of the thyroid hormones.

[004] Thyroid disorders are common and include hyper- and hypothyroidism. Hypothyroidism is typically characterized by an elevated level of TSH, but varies widely in its clinical presentation. Furthermore, while some patients present with obvious clinical

symptoms, others require the use of biochemical tests to determine the status of thyroid function. As a result, hypothyroidism is generally considered to be under diagnosed. In recent years, a number of hypothyroid syndromes with subtle presentations have been identified. Subclinical hypothyroidism refers to a condition marked by normal levels of T4 and T3 with elevated TSH. "Euthyroid sick syndrome" and "low T3 syndrome" refer to a condition where low serum levels of T3 are present but normal TSH and T4 levels are observed. These conditions have been associated with a number of nonthyroidal illnesses including congestive heart failure, clinical depression, mood disorders. Whether thyroid hormone replacement therapy is efficacious in the treatment of such disorders remains to be established.

[005] Hypothyroidism is the most common disorder of the thyroid and is manifested through the thyroid gland's inability to produce sufficient thyroid hormone, primarily 3, 3', 5-triiodothyronine (also known as T3). Symptoms associated with hypothyroidism include cold intolerance, lethargy, fatigue, chronic constipation and a variety of hair and skin changes. Although none of these conditions are life threatening, the disease, left untreated, could result in myxedema, coma, or death.

[006] The cause of hypothyroidism in the U.S. is brought about by either autoimmune destruction of the thyroid tissue (Hashimoto's disease), ^{131}I therapy, or ablative surgery. It is estimated that 8 to 10 million people in the United States have low thyroid gland function, but only about 4 to 5 million hypothyroid cases have been diagnosed and treated. The prevalence of hypothyroidism increases with age, particularly within the female population. The modern history of thyroid medication starts in the 1890's when desiccated pig thyroid was used to treat hypothyroidism. Thyroxine (3,3', 5, 5'-tetraiodothyronine), also known as T4, was introduced over forty years ago as a means to deliver the T3 hormone slowly without subjecting the patient to a transient hyperthyroid state. A synthetic drug based on blending T3 with T4 in a biomimetic fashion was introduced as an improved version. The medical community has discouraged this regimen, however, due to its potential for life-threatening T3 spikes.

[007] More recently, SYNTHROID, a synthetic T4 compound, has captured more than 70% of the hypothyroidism market. SYNTHROID's sales are reported to be in excess of $500 million (with additional sales in the market being taken by generic versions of thyroxine).

[008] Although T4 is a safe and effective way to treat hypothyroidism, a potential problem exists. Sufficient data has been generated that shows that SYNTHROID has a relatively short shelf life. The FDA has recommended that manufacturers of thyroid drugs address this problem.

[009] U.S. Patent Number 5,225,204 is directed to improving the stability of levothyroxine sodium. This patent indicates that the stability of the levothyroxine is affected by the presence of some carbohydrate excipients, such as dextrose, starch, sugar, and lactose. This patent claims that stability is achieved through mixing the levothyroxine with a cellulose carrier, with or without the addition of either polyvinyl pyrrolidine (PVP) or a Poloxamer.

[010] U.S. Patent Number 5,955,105 is also directed to providing an improved, stable, solid dosage form of thyroid hormone pharmaceutical preparations. This patent claims pharmaceutical preparations of thyroxine drugs including a water-soluble glucose polymer and a partially soluble or insoluble cellulose polymer to provide the stability. The indicated stability is determined as an absence of potency loss when the preparation is stored at 40 degrees C and 75% relative humidity for six months. U.S. Patent Number 5,955,105 is hereby incorporated by reference, particularly for its teachings on components of and production of pharmaceutical preparations of thyroxine drugs.

[011] It has been reported that the major product of T4 decomposition is diiodotyrosine (DIT). Latham, et al., showed that T4 in the blood decomposes into quinone-containing molecules. Both of these reports lead to the conclusion, heretofore unreported, that the pathway for T4 decomposition goes through a hydrolysis step with the loss or cleavage of an iodide. The energy required to cleave an sp^2-hybridized iodide-carbon bond, as in the case of T4, is not available under ambient conditions. As shown in Figure 1, tautomerization prior to hydrolysis is required in order for T4 to decompose into

DIT and iodoquinone. In other words, the spontaneous tautomerization of T4 is the "trigger" for its decomposition.

[012] The energy required to cleave a phosphorus-oxygen bond is greater than that for breaking a hydrogen-oxygen bond. The present invention prevents the tautomerization by replacing the hydrogen on the phenolic hydroxyl with a phosphinate group. By preventing tautomerization (as shown in Figure 3), the hydrolysis step cannot take place, thereby, reducing the lability of T4 to hydrolysis and increasing its shelf life.

[013] The dimethylphosphinate group has been used as a protecting group for tyrosine in peptide synthesis. Ueki *et al.*, *Tetrahedron Letters 27(35)*:4181-4184 (1986). The purpose of this group, however, was only to offer protection during peptide synthesis. It was not used to stabilize any other compounds, and was used only as part of a step during synthesis, never as a compound for use as a pharmaceutical composition. One aspect of the present invention is that it was not heretofore appreciated that stabilization was necessary in thyroid hormones. This problem is herein both recognized and solved by the use of phosphinate (and possibly other) protecting groups on the phenolic hydrogen. The resulting protected hormone is not deprotected *in vitro*. Rather, the hormone or hormone precursor is ingested while still protected by the phosphinate group.

[014] The result of the present invention is a prodrug to a thyroid hormone, which will be converted to the thyroid hormone *in vivo* after being provided to the patient in a treatment regimen. The prodrug will be hydrolyzed in the stomach or the gut into thyroxine and the biologically inert dimethylphosphinic acid. This provides a drug with all the therapeutic advantages of SYNTHROID with the additional advantage of increased stability, i.e., longer shelf life. Another advantage is that a thyroxine product with increased stability will be useful in producing either an injectable product or oral dosage forms such as tablets, capsules, solutions and oral suspensions (suitable for children) which are desirable. Yet another advantage of the present invention is a method to stabilize and increase the shelf life of thyroxine and related thyroid hormone compounds.

[015] T3 is metabolically active via binding nuclear thyroid hormone receptors and modulating transcription of specific genes. T4 is far less active in the regulation of

transcription and is generally considered a prohormone. The metabolic effects of T4 result from the conversion of T4 to T3 by deiodinase enzymes in peripheral tissues, and at the subcellular level once T4 enters a target cell. As noted previously, the T3 in circulation is largely the result of T4 to T3 conversion in the liver.

[016] The use of the active hormone, T3, as replacement therapy in hypothyroid conditions has met with limited success primarily because occasionally rapid increases in serum concentrations, or "spiking" levels, of this hormone in the serum occur, which could prove dangerous to patients whose cardiac status is compromised. For this reason, therapy with the prohormone, T4, has become the treatment of choice in hypothyroidism since, to be active, it first must be converted to T3, *in vivo*, a process which eliminates the potential for spiking T3 serum levels and any serious sequela. However, recent studies of T4 suggest that a general decline in a patient's ability to convert T4 to T3 is associated with aging, and also has been observed where stress or concurrent disease is present. Additionally, a deficiency in the T4 to T3 conversion capacity of particular organs or organ systems may exist. Given the problems associated with the use of either T3 or T4 as thyroid hormone replacement as herein identified, there is a need for an efficient, effective, low-cost and readily available mechanism for the delivery of thyroid hormones and derivatives thereof. Further, there is a need for compositions and methods to treat hypothyroid conditions and control the absorption of T3 *in vivo*.

[017] The compounds of the present invention may be provided in several useful forms, including pharmaceutical compositions in the form of ingestable tablets, capsules, oral solutions and suspensions, or intravenous solutions.

SUMMARY OF THE INVENTION

[018] One embodiment of the invention comprises an iodothyronine compound protected at the phenolic hydroxyl with a protecting group. In a preferred embodiment the iodothyronine compound is a triiodothyronine protected at a phenolic hydroxyl with a protecting group said protecting group selected from the group consisting of dialkylphosphinate, diarylphosphinate, alkylarylphosphinate, dialkylphosphate, diarylphosphate, aklylarylphosphate, acetyl, trialkylsilyl, and benzyloxy carbonyl. In

another embodiment the iodothyronine compound is reverse triiodothyronine. In another embodiment the iodothyronine compound is thyroxine. In another embodiment the iodothyronine compound is 3,5 diiodothyronine. In another embodiment the 3,5 diiodothyronine is selected from 3,5 diiodothyronine, 3',5' diiodothyronine, and 3,3' diiodothyronine. In another embodiment the iodothyronine compound is 3-monoiodothyronine.

[019] In one embodiment the dialkylphosphinate or dialkylphosphate is a C_1 to C_{18} substituted alkyl, more preferably a C_1 to C_8 substituted alkyl, and more preferably a C_1 to C_4 substituted alkyl. In another embodiment the dialkylphosphinate or dialkylphosphate is a C_1 to C_{18} unsubstituted alkyl, more preferably a C_1 to C_8 unsubstituted alkyl, and more preferably C_1 to C_4 unsubstituted alkyl. In a preferred embodiment the dialkylphosphinate is a diethyl or a dimethylphosphinate. In another preferred embodiment the dialkylphosphate is a diethyl or a dimethylphosphinate. In another embodiment the diarylphosphinate or the diarylphosphate is a substituted phenyl or an unsubstituted phenyl.

[020] In another embodiment the protected iodothyronine compounds of the invention are further combined with pharmaceutically acceptable excipients. In a preferred embodiment the iodothyronine compositions are utilized in a method of treating disorders related to improper thyroid function. More preferably, the condition is related to depression, hypothyroidism, mood disorders, general loss of thyroid function due to aging, or autoimmune destruction of the thyroid tissue. Most preferably, the condition being treated is hypothyroidism.

[021] In another embodiment, the protected iodothyronine compounds described above provide increased stability and stabilization of the thyroid hormone compared to thyroid hormone which is not protected at the phenolic hydroxyl, resulting in an increase in shelf life.

[022] In a preferred embodiment, the protected iodothyronine compounds are in an oral dosage form suitable for oral administration. Preferred oral dosage forms are tablets, capsules, oral solutions, and oral suspensions. In another embodiment the protected iodothyronine compounds are in the form of an intravenous preparation.

[023] The protected iodothyronine compounds prevent tautomerization from occurring at the phenolic hydroxyl and therefore the hydrolysis is prevented or delayed. By preventing the hydrolysis the shelf life and stability of the iodothyronine compound is increased.

BRIEF DESCRIPTION OF THE DRAWINGS

[024] Figure 1 shows the pathway of the degradation of T4. Following tautomerization of T4, the intermediate is subjected to hydrolysis, to produce DIT and a quinone.

[025] Figure 2 is the dimethylphosphinate of T4, thyroxinyldimethylphosphinate.

[026] Figure 3 shows the phosphinate-protected T4 and its inability to tautomerize.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[027] The present invention is practiced by using phosphinate protecting groups to protect against the decomposition of thyroxine and related compounds. These related compounds are preferably other iodothyronines, such as triiodothyronine (T3), 3,5-diiodothyronine (3,5-T2), 3,3'-diiodothyronine (3,3'-T2), reverse triiodothyronine (3,3',5'-triiodothyronine, rT3), and 3-monoiodothyronine (3-T1). The related compounds are also meant to include amino acids such as thyronine, diiodotyrosine, and iodotyrosine, and may include any amino acid that is unstable in the presence of trace amounts of water.

[028] To practice this invention, thyroxine or a related compound is reacted with a dialkyl- or diaryl-phosphinate compound, such as a dialkyl- or diaryl-phosphinic chloride. Most preferably, the dialkyl group is dimethyl or diethyl. The alkyl group may also be any hydrocarbon, preferably C_1 to C_{18}, comprising either straight chained, branched chained, or cyclic compounds, optionally substituted with oxygen-, phosphorus-, sulfur- and nitrogen-containing groups. The aryl group may be any aromatic group, preferably phenyl, and may be optionally substituted with alkyl or additional phenyl groups, and may also be optionally substituted with oxygen-, phosphorus-, sulfur- and nitrogen-containing groups. The two

alkyl groups may be the same or different. There may also be one alkyl and one aryl group on the phosphinate. The dimethyl can be replaced with diphenyl, diethyl or any other dialkyl and get the same level of protection on T4. In addition, the phosphinate group can be replaced with a similarly substituted dialkyl-, diaryl-, or alkylaryl- phosphate group. Other groups that can be used instead of the phosphinate group include acetyl, trialkylsilyl, and benzyloxy carbonyl.

[029] "Hydrocarbyl" shall refer to an organic radical comprised of carbon chains to which hydrogen and other elements are attached. The term includes alkyl, alkenyl, alkynyl and aryl groups, groups which have a mixture of saturated and unsaturated bonds, carbocyclic rings and includes combinations of such groups. It may refer to straight-chain, branched-chain cyclic structures or combinations thereof.

[030] "Aryl" shall refer to aromatic groups which have at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

[031] "Carbocyclic aryl groups" shall refer to groups wherein the ring atoms on the aromatic ring are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups and optionally substituted naphthyl groups.

[032] "Monocyclic carbocyclic aryl" shall refer to optionally substituted phenyl, being preferably phenyl or phenyl substituted by one to three substituents, such being advantageously lower alkyl, hydroxy, lower alkoxy, lower alkanoyloxy, halogen, cyano, trihalomethyl, lower acylamino, lower amino or lower alkoxycarbonyl.

[033] "Optionally substituted naphthyl" shall refer to 1- or 2-naphthyl or 1- or 2-naphthyl preferably substituted by lower alkyl, lower alkoxy or halogen.

[034] "Heterocyclic aryl groups" shall refer to groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl, and the like, all optionally substituted.

[035] "Optionally substituted furanyl" shall refer to 2- or 3-furanyl or 2- or 3-furanyl preferably substituted by lower alkyl or halogen.

[036] "Optionally substituted pyridyl" shall refer to 2-, 3- or 4-pyridyl or 2-, 3- or 4-pyridyl preferably substituted by lower alkyl or halogen.

[037] "Optionally substituted thienyl" shall refer to 2- or 3-thienyl, or 2- or 3-thienyl preferably substituted by lower alkyl or halogen.

[038] "Biaryl" shall refer to phenyl substituted by carbocyclic aryl or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring, advantageously para.

[039] "Aralkyl" shall refer to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl, picolyl, and the like, and may be optionally substituted.

[040] "Lower" referred to herein in connection with organic radicals or compounds respectively defines such with up to and including 7, preferably up to and including 4 and advantageously one or two carbon atoms. Such groups may be straight chain or branched.

[041] The terms (a) "alkyl amino", (b) "arylamino", and (c) "aralkylamino", respectively, shall refer to the groups --NRR' wherein respectively, (a) R is alkyl and R' is hydrogen or alkyl; (b) R is aryl and R' is hydrogen or aryl, and (c) R is aralkyl and R' is hydrogen or aralkyl.

[042] The term "acyl" shall refer to hydrocarbyl-CO-- or HCO--.

[043] The terms "acylamino" refers to RCONCR)-- and (RCO_2 N- respectively, wherein each R is independently hydrogen or hydrocarbyl.

[044] The term "hydrocarbyloxycarbonyloxy" shall refer to the group ROC(O)O- wherein R is hydrocarbyl.

[045] The term "lower carboalkoxymethyl" or "lower hydrocarbyloxycarbonylmethyl" refers to hydrocarbyl-$OC(O)CH_2$ - with the hydrocarbyl group containing ten or less carbon atoms.

[046] The term "carbonyl" refers to -C(O)-.

[047] The term "carboxamide" or "carboxamido" refers to --$CONR_2$ wherein each R is independently hydrogen or hydrocarbyl.

[048] The term "lower hydrocarbyl" refers to any hydrocarbyl group of ten or less carbon atoms.

[049] The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched chain and cyclic groups.

[050] The term "alkenyl" refers to unsaturated hydrocarbyl groups which contain at least one carbon-carbon double bond and includes straight-chain, branched-chain and cyclic groups.

[051] The term "alkynyl" refers to unsaturated hydrocarbyl groups which contain at least one carbon-carbon triple bond and includes straight-chain, branched-chain and cyclic groups.

[052] The term "methylene" refers to -CH_2-.

[053] The term "alkylene" refers to a divalent straight chain or branched chain saturated aliphatic radical.

[054] The term "oxy" refers to -O- (oxygen). The term "thio" refers to -S- (sulfur). "Disulfide" refers to -S-S-.

[055] Although N-protection may not be necessary, the best yield of throxinyldialkylphosphinate is achieved by first protecting the nitrogen of T4 or related compound. Any method of protecting the nitrogen of an amino acid group known in the art may be employed in protecting the nitrogen of thyroxine and related compounds. Most preferably, the reagent of choice is trimethylsilylethoxycarbonyloxysuccinimide. The N-protected T4 is then treated with dimethylphosphinic chloride or diphenylphosphinic chloride, as before, and the product of this reaction is N-deprotected by treatment with trifluoroacetic acid. Deprotection can also take place using other mild acids, as well.

[056] The invention thus provides a method to stabilize and increase the shelf life of thyroxine and related thyroid hormone products. The compositions of the present invention will be used in methods of treating hypothyroidism, depression and other related dysfunctional thyroid hormone conditions. These products will be used at levels similar to

those used in treating hypothyroid patients with SYNTHROID. Determining the precise levels to be used in a particular patient may be accomplished using methods well known to those of skill in the art, including monitoring the levels of thyroid hormones in the blood using known techniques and adjusting the dosage accordingly to get blood levels within acceptable limits. The compositions will be particularly useful in providing injectable and oral suspension formulations, as well as tablets, for thyroid hormones.

[057] The present compounds can be administered by a variety of routes and in a variety of dosage forms including those for oral, rectal, parenteral (such as subcutaneous, intramuscular and intravenous), epidural, intrathecal, intra-articular, topical and buccal administration. The dose range for adult human beings will depend on a number of factors including the age, weight and condition of the patient and the administration route.

[058] For oral administration, fine powders or granules containing diluting, dispersing and/or surface-active agents may be presented in a draught, in water or a syrup, in capsules or sachets in the dry state, in a non-aqueous suspension wherein suspending agents may be included, or in a suspension in water or a syrup. Where desirable or necessary, flavouring, preserving, suspending, thickening or emulsifying agents can be included.

[059] Other compounds which may be included by admixture are, for example, medically inert ingredients, e.g. solid and liquid diluent, such as lactose, dextrose, saccharose, cellulose, starch or calcium phosphate for tablets or capsules, olive oil or ethyl oleate for soft capsules and water or vegetable oil for suspensions or emulsions; lubricating agents such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene glycols; gelling agents such as colloidal clays; thickening agents such as gum tragacanth or sodium alginate, binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinylpyrrolidone; disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuff; sweeteners; wetting agents such as lecithin, polysorbates or laurylsulphates; and other therapeutically acceptable accessory ingredients, such as humectants, preservatives, buffers and antioxidants, which are known additives for such formulations.

[060] Liquid dispersions for oral administration may be syrups, emulsions or suspensions. The syrups may contain as carrier, for example, saccharose or saccharose with glycerol and/or mannitol and/or sorbitol. In particular a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolize to glucose or which metabolize only a very small amount to glucose. The suspensions and the emulsions may contain a carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose or polyvinyl alcohol.

[061] Suspensions or solutions for intramuscular injection may contain, together with the active compound, a pharmaceutically acceptable carrier such as sterile water, olive oil, ethyl oleate, glycols such as propylene glycol and, if desired, a suitable amount of lidocaine hydrochloride. Solutions for intravenous injection or infusion may contain a carrier, for example, sterile water that is generally Water for Injection. Preferably, however, they may take the form of a sterile, aqueous, isotonic saline solution. Alternatively, the present compounds may be encapsulated within liposomes. The present compounds may also utilize other known active agent delivery systems.

[062] The present compounds may also be administered in pure form unassociated with other additives, in which case a capsule, sachet or tablet is the preferred dosage form.

[063] Tablets and other forms of presentation provided in discrete units conveniently contain a daily dose, or an appropriate fraction thereof, of one of the present compounds. For example, units may contain from 5 mg to 500 mg, but more usually from 10 mg to 250 mg, of one of the present compounds.

[064] It will be appreciated that the pharmacological activity of the compositions of the invention can be demonstrated using standard pharmacological models that are known in the art. Furthermore, it will be appreciated that the inventive compositions can be incorporated or encapsulated in a suitable polymer matrix or membrane for site-specific delivery, or can be functionalized with specific targeting agents capable of effecting site specific delivery. These techniques, as well as other drug delivery techniques are well known in the art.

[065] The invention will now be illustrated by, but is not intended to be limited to, the following examples.

EXAMPLES

Example 1: Preparation of 2-trimethylsilylethyl carbonochloridate (Teoc-Cl)

[066] To a solution of 2-trimethylsilylethanol (5.0 g, 42.3 mmol) in dichloromethane (35 mL) at 0°C was added triethylamine (4.7 g, 46.5 mmol). To this stirred solution was added dropwise a solution of triphosgene (4.40 g, 14.8 mmol) in dichloromethane (15 mL); a white precipitant was formed immediately. The mixture was stirred at low temperature for 15 minutes, the ice bath removed, and the mixture was stirred for an additional 1 hour at room temperature. After 1 hour, the white precipitant was filtered and washed with dichloromethane (~60 mL). The combined filtrate and washings were concentrated. The resultant oily carbonochloridate was used without further purification.

Example 2: Preparation of 1- [2(Trimethylsilyl)ethoxycarbonyloxy]pyrrolidin-2,5-dione (Teoc-0Su)

[067] 2-Trimethylsilylethyl carbonochloridate (4.8 g, 26.9 mmol) was taken up in dry acetronitrile (50 mL). The solution was cooled to 0° and solid N-hydroxysuccinimide (4.0 g, 34.8 mmol) was added with vigorous stirring followed by a solution of dry triethylamine (3.2 g, 31.6 mmol) in dry acetonitrile (5 mL). The mixture was stirred at low temperature for 15 minutes, then at room temperature overnight. The mixture was poured into water (200 ml) and extracted with ether (4 x 50 mL). The organic extracts were combined, washed with water (2 x 60 mL), 1 normal hydrochloric acid (60 mL), again water (60 mL), brine (60 mL), dried with magnesium sulfate and evaporated to dryness. The residue was taken up in boiling hexane (200 mL) and the solution allowed to cool. Crystallization was completed by storage at -15°C (yield: 1.70g).

Example 3: Preparation of N-Trimethylsilylethoxycarbonylthyroxine (Teoc-T4)

[068] To a stirred suspension of thyroxine (1.66 g, 2.14 mmol) in DMSO (15 mL) was added triethylamine (3.21 mmol) followed by solid Teoc-0Su (610 mg, 2.35 mmol). The mixture was stirred at room temperature overnight then diluted with water (22 mL), acidified with saturated potassium hydrogen sulfate solution and extracted with ether (3 x 45 mL). The combined organic extracts were washed with water (4 x 45 mL), dried with magnesium sulfate, and evaporated to dryness. (Yield: 1.87g).

Example 4: Preparation of [N-Trimethylsilylethoxycarbonyl-O-throxinyl]-dimethylphosphinate

[069] N-Trimethylsilylethoxycarbonylthyroxine (307 mg, 0.334 mmol) was dissolved in 10-mL dry chloroform, and to the stirred solution was added anhydrous triethylamine (154 μL, 1.10 mmol). After stirring for 10 minutes at room temperature, dimethylphosphinyl chloride (112.7 μL, 1.00 mmol) was added and stirring was continued at room temperature. After 90 minutes, the reaction appeared to be nearly completed by TLC analysis (chloroform/*i*-propanol/acetic acid, 85:10:5), based on relatively clean conversion of starting material (R*f* 0.34) to product (R*f* 0.22). The reaction was quenched by the addition of 20 mL 0.5 N HCl. The product was extracted into chloroform (3 x 30 mL). The combined chloroform layers were washed with brine, dried over magnesium sulfate and evaporated to dryness, affording 280 mg [N-trimethylsilylethoxycarbonyl-O-throxinyl]dimethylphosphinate (84% yield).

Example 5: Preparation of O-Thyroxinyldimethylphosphinate

[070] [N-Trimethylsilylethoxycarbonyl-O-thyroxinyl]dimethylphosphinate (42 mg, 0.042 mmol) was dissolved in 1.5 mL of trifluoroacetic acid. After 5 minutes stirring at room temperature, TLC analysis (chloroform/*i*-propanol/acetic acid, 85:10:5) showed the deprotection to be complete. The solvent was removed by rotary evaporation. Azeotropic evaporation with hexane afforded the product as a fine, white powder in nearly quantitaive

yield. NMR analysis showed the product to be the desired O-thyroxinyldimethylphosphinate.